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# **Reply on RC1**

Laura Anahí Macario-González et al.

Author comment on "Geodiversity influences limnological characteristics and freshwater ostracode species distributions across broad spatial scales in the northern Neotropics" by Laura Anahí Macario-González et al., Biogeosciences, https://doi.org/10.5194/bg-2021-298-AC1, 2022

**Reviewer 1.-** We deeply appreciate all your comments and suggestions to improve the manuscript, they contribute significantly to improve the manuscript. Below you will find answers for each of your comments and questions. In case we were unable to follow them, we provide an explanation.

General comments

Parts of the manuscript are difficult to understand mainly because its language is often not precise enough, the sentence structure is confusing and the grammar is erroneous. Understandability could be improved by reducing different terms for the same purpose, non-biotic/environment, examples are principle component/dimension, elevation/altitude, orography/topography, limnological (sub)regions/limnological (sub)groups, aquatic systems/aquatic ecosystems/aquatic environments/lakes, etc. Some examples of confusing sentence structures and grammatical errors are given in the specific comment section; however, many more issues will remain unlisted and should be edited by the authors, potentially with the help of a native speaker or proofreading service.

Answer: The relevance and usage of the terms in the manuscript was reviewed. Those terms that cause confusion are changed and homogenized to improve readability. Sentence structure and grammatical errors are corrected by a native English speaker with appropriate scientific background.

The next issue is partly a consequence of the previous issue but not solely: The way how the SEM was set up and how the authors derived their final model is diffuse and not well explained. The parameters which are used to describe geodiversity and limnology, the choice of exogenous and endogenous variables as well as some relationships described by the SEM are not intuitive. For example, (i) in model 2 (Sect S1 in the Supplement), geodiversity (latent variable) is described only by elevation and latitude (observed variables), despite available parameters about the type of bedrock and the mineral composition of lake sediments; (ii) the usage of altitude as endogenous variable (i.e. variables that are dependent in at least one equation), (iii) certain paths

in the SEM such as the effect of geodiversity on altitude, the effect of limnology on conductivity, although conductivity was used as parameter describing limnology throughout the manuscript, the effect of conductivity on species diversity but not on species associations and the effect of altitude on species associations but not on diversity

(see Fig. 6, further obscurities are addressed in the specific comment section). To improve SEM sections in the manuscript, it needs (i) a more consistent usage of terms normally used in the context of SEM (i.e. exogenous, endogenous, latent, observed variables), (ii) a rework of Fig. 6 which should include observed variables describing latent variables (geodiversity and limnology), (iii) a construction of paths in the SEM based on clearly stated hypothesis and (iv) a more straightforward selection of observed variables to describe latent variables (limnology, geodiversity). The latter could be achieved for example by using environmental variables which were forward selected in the CCA, which was used to examine effects of environment on species composition.

Answer: SEM analysis was reviewed and re-description of the rationale behind variable selection was conducted with a detailed explanation of the observed variables describing latent variables, as well as exogeneous-endogenous variable selection. We followed the four directions suggested by the reviewer and therefore new paths are explained. Detail discussion of the reviewer questions is included in both results section of the manuscript and section 1 of the supplementary material. Geodiversity resulted the most important variable explaining limnological patterns and in turn, limnology to species composition (distribution). The metrics of fit of all models are presented in supplementary material.

# Specific comments

# Abstract

32: What exactly do you mean with the term "biological composition"? In Fig 6 you use the terms species diversity and species associations. According to Fig 6., the effect of limnology on species associations is not significant, the direct effect of elevation (in Fig 6 called altitude) on species diversity is not tested.

Answer: The term biological composition was reviewed because it introduces confusion to the reader. We add definitions of the terms used, particularly geodiversity, biological composition and limnology. Limnology is not significant for species, except when their indirect influence is evaluated. Elevation is also directly tested and results are relevant for species associations.

33: From which result do you derive that geodiversity is the most important driver? Geodiversity is a fundamental driver because it shapes limnology. Hence, I consider geodiversity on a different hierarchical level and difficult to compare to limnology (exogenous vs endogenous variable).

Answer: The PCA and the SEM reveal that geolimnology is the most important variable differentiating limnological regions. Here, we assumed that limnology and elevation (with its indirect effect on species diversity) are a function of geodiversity, and given that they better explain the ostracode association, they are assumed as most important drivers. However, following the view of the reviewer, we are considering the effect of geodiversity at different hierarchical level such as the individual influence over limnology and species and we re-discus the results.

# Introduction

45 is difficult to understand: What do you mean with "biodiversity is dynamic" and "faster rates"; faster than what?

Answer: We clarified the text and modified as follows: "Biological diversity, defined as the variety of life forms in a place on Earth (Huston, 1995), is strongly related to geodiversity, as species are distributed in response to landscape features. Biological diversity is dynamic. Species evolve and distribution patterns change at rates different from rates of change of geodiversity"

61: it is unclear what you mean with "biological structure"

Answer: To avoid confusion, we changed here the term "biological structure" by "biogeographical patterns". All terms used to refer to biological systems were homogenized.

71: Confusing sentence structure

Answer: corrected, all manuscript will be revised by a native English speaker

80: Although the study is very comprehensive, it would greatly benefit from an additional layer consisting of data about land use/human activity

Answer: We consider that this information can greatly benefit our study, now we do not have land use data for all our study sites. Evaluate land use/human activities is beyond of the scope of this study, as we analyze the relationships between geology (geodiversity), limnology and species composition. Indirectly, we analyze the effect of human impacts on aquatic ecosystems using secchi depth, and other trophy state variables. In the conclusions, we add a paragraph of future directions for this study, and particularly describing the importance of the influence of land use and human activity on aquatic ecosystems and biological change.

88: Confusing sentence structure

Answer: corrected, same comment than in line 71.

94: ostracods are a well-suited group

Answer: corrected according to the reviewer suggestion

94: The study is not investigating traits.

Answer: following the view of the reviewer we modify the term

94: singular: ostracod, plural: ostracods. Rephrased sentence: Ostracods are bivalved microcrustaceans which are abundant, diverse and widely distributed in recent environments.

Answer: changed according to reviewer suggestion

103: You are also looking at effects on species diversity.

In general, terms like diversity, composition, assemblages, associations, species distribution, biological structure, are not well defined and often used synonymously. To avoid confusion, please stick to the same expression throughout the manuscript if the purpose is the same.

Answer: We carefully verify all terms and those used inappropriate were changed and homogenized to avoid confusion. We provide a clear definition for biogeographic terms used.

# Material and Methods

156: Here you use "non-biological" and in other parts "environmental", I suggest to stick to either "environmental" or "abiotic" throughout the entire manuscript, also in figures.

Answer: We used "environmental" instead of "non-biological", throughout the manuscript and figures

186: How did you handle missing data?

Answer: for statistical purposes, missing data were completed with average values of the respective variable, as missing data represented less than 8% of the dataset. This information is included in the manuscript.

200: The maps do either not represent the measured data or it is not visible. Please add the measured data. Also, a reference to the figure is missing.

Answer: Maps were re-designed and measured data were integrated to the interpolation map to clearly visualize the power of prediction. We also added the reference for the figure.

218: Clarify how you distinguished species groups. Was it manually done by visually examining the graph?

Answer: For species group determination we apply a hierarchical cluster analysis based on Ward distances and then overlapped (hclust in R) on the NMDS ordination. These techniques usually validate one to another and reduce the uncertainty for group boundaries determination. We describe this procedure in the manuscript.

221: Here you use "relating non-biological variables" and later in the paragraph "environmental variables", take care of consistency.

Answer: corrected throughout the text, we used environmental variable.

221: Besides geological effects, you also assessed limnological effects (temperature, conductivity, etc.)

Answer: corrected, we included a paragraph on the limnological effect

231: What do you mean with "related environmental variables"?

Answer: We are referring to the variables that derived or are influenced by geology, namely, sediment geochemistry and elevation. To clarify the sentence, we excluded the phrase "related environmental variables" and those considered in the analysis were enlisted.

231: You also assessed the influence of geodiversity on species diversity not only on the composition (in Fig 6 called "species association").

Answer: This is correct, we included it in the text to clarify methods

235: It is not clear that you use elevation gradients, latitude and bedrock as observed variables to explain geodiversity (latent variable). The same applies for limnology and its observed variables.

Answer: Please see the answer to general comments.

236: It is not clear if geodiversity is assumed as indirect, direct or both, the same for limnology

Answer: Geodiversity and limnology was evaluated as both exogenous variables and their direct and indirect influence on species composition and richness was tested. Please see section 1 of supplementary material.

237: It is not clear, how you take vulcanism, precipitation and marine-freshwater interactions into account and where the major anion and cations belong to in the SEM. Answer: Please see general comments for SEM

#### Results

Results are normally written in simple past tense

Answer: The text was reviewed by native American scientist.

253: Here and in Table 1 the term "(sub)groups" is used, in the text mainly

"sub(regions)". In general, I think the terms "limnological classification" and "limnological regions" are confusing as you also use the term "limnology" as hypernym for water chemicals and physical properties of the aquatic ecosystems. The "limnological classification", however, is based next to limnological variables also on geological, sedimentological and mineralogical variables.

Answer: We homogenized the term and use sub-regions, the term sub-groups was deleted to avoid confusion. Also, the term limnological was defined to be more precise.

Fig 2 (c): It is difficult to track dots to site abbreviations. Also, site 65 appears two times once with the site abbreviation CHI and once with CH1

Answer: we clarify and better link dots with site abbreviations, however, because of the image size, we refer to figure 1 to identify site abbreviations. The site 65 which was duplicated is corrected.

271: Are the "thirteen variables" those which were selected based on "superimposed arrows"? Please clarify

Answer: Yes, all statistical analyses were conducted using a data base with uncorrelated variables, those represented by superimposed arrows in the PCA ordination were deleted, as they demonstrated similar response. This procedure was described in detail in methodology and in the results sections.

Fig 3 and 4 (b-d): Could you show sites with colours according to the observed values to see how well they fit into the predicted surface. Write the variables which are mapped in the graphs also in the legend or put them as title.

Answer: We added measured values to the predicted map to verify the prediction power of the algorithm. We also added the variable name on the legend and as title to facilitate the visual recognition.

300: You write about "sedimentology and geology" as important variables. However, there are no variables called like this. In order to make that point clear, I think it would help, if parameters in Fig 3 (a) and Fig 4 (a) are coloured according to their type (i.e. limnology, sedimentology, geology, mineralogy). This would also help to not confound carbonate measurements derived from the water with measurements derived from the sediment.

Answer: We appreciate this observation; we conducted the recommendation and figure 3 (a) and 4 (a) were modified.

358: "supporting NMDS ordination" or supporting group selection?

Answer: supporting group selection is correct; the text was changed accordingly.

361: Are you deriving the tolerance to high conductivity from the literature or from your CCA? If the latter is true, you should refer to Fig. S3.

Answer: We derived high conductivity tolerance from the results of our analysis, and we refer to figure S3.

397: In S1 you mention five models instead of six.

Answer: We corrected accordingly

Also, in S1 you write "... we assume that **elevation gradients**, **bedrock** and **latitude** were primary factors determining biological composition in aquatic systems. These three factors were then used as exogenous variables..." Here you state that initially **geodiversity**, **limnology** and **elevation** were your three exogenous variables. It is not clear which variables describe geodiversity and limnology. Answer: This is a terminology issue, latitude was associated to limnology because of ionic composition of waters (expected to be affected by precipitation); bedrock was associated to geodiversity. Terminology issues are corrected to exclude such confusion

408: Why do you test the influence of species associations on diversity? What is the hypothesis?

Answer: The analysis of species association on diversity was excluded.

408: Why "indirect" when there is a "direct" link from limnology to species associations?

Answer: Here we consider that the confusion is an issue of the wide variety of terms used in the text. In this case, we changed to direct effect.

Fig. 6: instead adding "environmental variables" to limnology, add observed variables which describe limnology, the same applies for geodiversity. Why are you not looking at the direct effect of limnology on species diversity?

Answer: Please see our answer to general comments on SEM

# **S1**

In general, it is a good idea to provide details about all SEMs, however, the text in S1 often explains the same as the main text, but in a different way, which adds to the confusion.

Paragraph 3: "Geodiversity was constructed only with elevation and latitude as predictors, whereas limnology only with conductivity. The selection of these variables resulted from the fact that elevation was directly related with water temperature in lakes and latitude with presence of carbonates given reduction in precipitation and increase evaporation" I don't understand why elevation and latitude were used as observed variables for geodiversity. First of all, if elevation was related to water temperature and latitude to the presence of carbonates, why not take water temperature and carbonates as observed variables, instead of related variables. But secondly, water temperature was always part of the variables describing limnology and not geodiversity.

Paragraph 4: Model 4 "was constructed on the basis of the model 2 and 3 with respect of predictors of geodiversity and limnology". It is not clear, which observed variables are actually used for geodiversity and limnology.

Answer: We appreciate all these questions related to the model design, a critical analysis was conducted taking into account all of them. Please see general comments to the SEM.

# Discussion

419: What do you mean with "Geology and associated variables"?

Answer: "associated variables" in this case, are those which are directly influenced by geology such as mineralogy, sediment geochemistry and elevation. In order to avoid confusion, we excluded the term "associated variables" and explicitly describe them.

493: Your results show a different picture, see line 277: "pH was highly correlated (>0.73) with the second component (PC2), suggesting that it is the second most influential variable of the YG aquatic environments (Fig. 3a, Table S2.1)"

Answer: we consider that the text is congruent with the figure, however, we found the term "second most influential variable" confusing, and we will modify the text accordingly.

# Chapter 4.1. is well written

511: I suggest to state this more carefully as you are only looking at a handful of lakes without applying any statistical analysis to test this pattern.

Answer: We agree that we are over-generalizing some of our interpretations such as in this case. We will carefully check this to avoid inaccurate assumptions. We consider that pointing out what the results are covering will very much improve the manuscript. For example, instead of using aquatic communities, we will use ostracods (the target group).

539: The obvious spatial pattern of species composition may also be a hint to dispersal related processes which are not at all touched in this study. A potential statistical way to incorporate spatiality in this study would be to include space as exogenous latent variable in the SEM with latitude and longitude as observed variables. The possibility of dispersal limitations acting as additional driver structuring ostracod communities should at least be discussed.

Answer: We appreciate this comment, and the topics suggested was included in our SEM analysis.

546: What is the indirect effect of limnology on species composition? In Fig 6. I see a direct effect of limnology on species associations (I guess this is meant with species composition), and a questionable (see other comments) indirect effect via conductivity on species diversity.

556: "elevation" is not used as a variable in the CCA

Answer: elevation is a driver of temperature, the term is corrected.

575: "as evolutionary trait"?

Answer: This issue is related with the grammatical errors conducted throughout the study, but inaccurate terms are being corrected.

# **Technical comments**

Answer: All technical comments are considered and included in the manuscript.



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# **Reply on RC2**

Laura Anahí Macario-González et al.

Author comment on "Geodiversity influences limnological characteristics and freshwater ostracode species distributions across broad spatial scales in the northern Neotropics" by Laura Anahí Macario-González et al., Biogeosciences, https://doi.org/10.5194/bg-2021-298-AC1, 2022

**Reviewer 2.-** We appreciate all the comments and suggestions, they significantly improve the manuscript. Below we provide answers to all comments. We have tried to address most of the suggestions made by reviewer 2, but in case this is not possible, we provide a detailed explanation.

Comment: Because the authors are evidently not native speakers of English, there are considerable numbers of grammatical mistakes and poorly-phrased passages, most of which are detailed below with suggestions for improvement.

Answer: Sentence structure and grammatical errors were corrected by a native English speaker with appropriate scientific background.

Comment: In several places the text is over-generalized, for example Line 35 which refers to "aquatic biological composition" – in fact, this study is only about ostracods.

Answer: We agree and modified the text accordingly to prevent over-generalization. We now refer only to freshwater ostracods throughout the manuscript and exclude the term aquatic biological composition. Ostracodes, however, are recognized as bioindicators of aquatic ecosystems and ecological interactions change. The group belongs to the base of trophic chain and changes on its composition is evidence of aquatic biological composition alteration. The same apply on interpretations of past environments, because ostracodes have abundant fossil record and they reveal changes in the biological composition in response of external variables. We also modify the title.

Comment: One area that requires clarification is the enumeratation of ostracods (Line 204). Apparently the investigators counted ostracods, but the details of counting aren't presented. How many per sample?

Answer: For this study, we counted specimens in a standard sample volume of 50ml, obtained from filtering 200 L of water in the littoral zones. Then, we used relative abundance for statistical analyses. All this information is described in detail in the manuscript. Count data expressed as relative abundance is provided in supplementary material https://doi.pangaea.de/10.1594/PANGAEA.940254

Comment: It's also not clear what they mean by "identified three adults" passage.

Answer: We used three well-preserved adult specimens for microdissection and an accurate identification down to species level. As the target specimens are microcrustaceans, identification based on a single specimen can be misleading because of morphological plasticity and mutations, identification based on at least three specimens are considered reliable. We clarified this sentence in the manuscript.

Comment: Analysis seems to be based on presence/absence, but if count data are available for each sample, then ordination can be based on square root-transformed percentage data.

Answer: Species assemblages were analyzed based on presence/absence data for the NMDS analysis. Because species composition from different sites (latitudes) are evaluated and most species are not shared between lakes, values of species richness and abundances are highly variable between lakes (highly dissimilar). The NMDS outcome largely depends on the distance-base index used, therefore, literature recommend using presence/absence data in cases when dissimilarity is relevant. Please see Ecology, 84(3), 2003, pp. 777–790 by the Ecological Society of America. For the CCA, both presence/absence and relative abundance data resulted in very similar ordination. In the first version of the manuscript, we present the CCA graph of presence/absence data to be homogeneous with NMDS analysis (both with Presence/absence data), but following the view of the reviewer, we present now the CCA graph based on count data.

Comment: A more serious shortcoming is data handling. Apparently the investigators did not test each variable for normality. Skewed data should be transformed to produce a more normal distribution prior to ordination. For a clear methodology I recommend the most-recent edition of Tabachnick and Fidell's book Using multivariate statistics.

Answer: Environmental data, given different units of quantification, were standardized prior to analysis by subtracting the mean value and dividing by standard deviation. Then, normality was assessed for each variable. Ordination and statistical analysis were therefore performed in a preprocessed database with data normal distributed. As this process may be unclear in the manuscript, we described it in detail. We appreciate the recommended literature.

Comment: The authors should be careful in discussing diversity, which is not the same as richness. Also, such comparisons are difficult to interpret because collection size varies among the studies that they cite. Using 'alpha diversity' and 'beta diversity' would help.

Answer: We used alpha diversity to estimate diversity in the region, when possible we used this index to compare our results with other studies conducted worldwide.

Comment: I suspect that the authors are correct, that geology (local bedrock, karst vs volcanic) is the main influence on ostracod distribution. It's not clear why they use the term 'geodiversity' when bedrock geology alone seems to be the main driver. Data on 'geodiversity', i.e. local diversity of geology (geology, geomorphology, hydrology) aren't presented for each of the lakes.

Answer: The influence of geodiversity on ostracode species distribution is most likely occurring at different hierarchical levels. For instance, we did not find evidence (in SEM analysis) that local bedrock alone explains the species distribution. The hypothesis postulated by reviewer was evaluated and discussed in the manuscript. We use the term "geodiversity" because we are providing site characteristics, such as elevation and bedrock type, which constitute part of the geodiversity. We do not provide detailed data on geomorphology and hydrology because this is beyond the scope of the study. We evaluated the relevance of the term "geodiversity" and we decided to continue using it.

Comment: Another driver seems to be understated, namely precipitation rates. Region YG is low rainfall, Region GSHN is generally higher rainfall. This correlates with karst/low elevation vs volcanic/highlands, but isn't rejected as a main driver. In order to conclude firmly that it's geology rather than rainfall, the authors should add another variable to their data set (i.e. mean annual rainfall at each lake).

Answer: The influences of annual precipitation, precipitation seasonality, air temperature seasonality on species composition was exploratory evaluated in the first stage of the study. This data was extracted from the Worldclim data base. Precipitation variables were, however, highly correlated with major ions and cations, particularly Mg and Ca. We decided to use the latter for the analysis, as their variability in lakes may explain more clearly and directly ostracode distributions. We also considered precipitation as a climatic variable which are out of the scope of the manuscript.

Specific comments –

Answer: We appreciate all these comments and all of them were included to the manuscript.